UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: 02-JUN-2011

Subject: **Prohexadione Calcium**. Section 3 Registration for Use on Sweet Cherries. **Summary of Analytical Chemistry and Residue Data.**

PC Code: 112600	**DP Barcode:** D384706
Decision No.: 437934	**Registration No.:** 7969-188
Petition No.: 0F7765	**Regulatory Action:** Section 3
Risk Assessment Type: NA	**Case No.:** 7030
TXR No.: NA	**CAS No.:** 127277-53-6
MRID No.: 48106701 thru -03	**40 CFR:** §180.547

From: George F. Kramer, Ph.D., Senior Chemist
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Risk Assessment Branch 1 (RAB1)
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Through: Dana M. Vogel, Branch Chief
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To: Tony Kish, RM 22
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Executive Summary

Prohexadione calcium is a plant growth regulator proposed for use in sweet cherry orchards that reduces vegetative growth and can reduce or delay the need for tree pruning. Prohexadione calcium works by inhibiting the biosynthesis of gibberellins, resulting in a decrease in cell elongation and a slowing of cell growth. Shoot reduction may cause positive benefits such as better light penetration, better fruit coloring, and better air flow within the tree. Bud and bloom production may be increased the following year, resulting in increased fruit production. The registered product, Apogee® Plant Growth Regulator (EPA Reg. No. 7969-188), is formulated as a wettable granule (WG) containing 27.5% prohexadione calcium.

Tolerances are currently established under 40 CFR §180.547(a) for residues of prohexadione calcium in/on pome fruit, peanut, grass, and livestock commodities at levels ranging from 0.05 to 3.5 ppm.

The proposed tolerance for residues of the plant growth regulator, prohexadione calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate), is:

Sweet Cherries .....0.50 ppm

Based on apple and peanut metabolism studies, the HED Metabolism Assessment Review Committee (MARC) concluded that for these crops parent compound is the only residue of concern for the tolerance expression and dietary risk assessment (Memo, G. Kramer, 4/07/00; DP# 263502). HED concludes that this decision may be translated to cherry.

A liquid chromatography with tandem mass spectrometry (LC-MS/MS) method (BASF Method 564/0) is available for the enforcement of the proposed tolerances for sweet cherries. HED has determined that BASF Method 564/0 is a suitable enforcement method for fruit commodities, as defined in Standard Operating Procedure (SOP) No. ACB-019 (9/15/08).

Adequate crop field trial data are available, provided the Apogee® label is modified to specify a preharvest interval (PHI) of 20 days. Based on the results of these trials, the appropriate tolerance for residues of prohexadione calcium in/on sweet cherry is 0.40 ppm. Revised Sections B and F are requested.

Regulatory Recommendations and Residue Chemistry Deficiencies

Pending submission of revised Sections B (see requirements under Directions for Use) and F (see requirements under Proposed Tolerances), there are no residue chemistry issues that would preclude establishment of an unconditional registration and the following permanent tolerances for the growth regulator, prohexadione calcium, including its metabolites and degradates, in or on the commodities in the table below. Compliance with the tolerance levels specified below is to be determined by measuring only prohexadione calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate), in or on the following commodity:

Cherry, sweet .....0.40 ppm

860.1200 Directions for Use

- The Apogee® label should be modified to specify a PHI of 20 days.

860.1550 Proposed Tolerances

- The petitioner should submit a revised Section F reflecting the HED-recommended tolerance expression, commodity definition, and residue level specified above.

A human-health risk assessment is forthcoming in a separate document.

Background

Prohexadione calcium is a plant growth regulator that works by inhibiting the biosynthesis of gibberellins, resulting in a decrease in cell elongation and a slowing of cell growth. The chemical structure and nomenclature of prohexadione calcium is presented in Table 1. The physicochemical properties of the technical grade of prohexadione calcium are presented in Table 2.

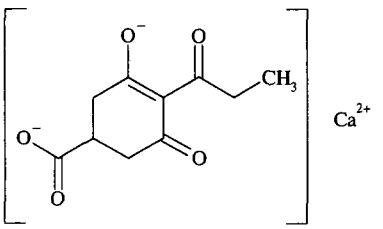
Table 1. Test Compound Nomenclature.	
Compound	
Common name	Prohexadione calcium
Company experimental name	BAS 125 W
IUPAC name	calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate
CAS name	calcium 3-oxido-5-oxo-4-propionylcyclohexa-3-enecarboxylate
CAS registry number	127277-53-6
End-use product (EP)	Apogee® Plant Growth Regulator (EPA Reg. No. 7969-188)

Table 2. Physicochemical Properties of Prohexadione Calcium.		
Parameter	Value	Reference
Water solubility (20°C for 96.6% pure sample)	174 mg/L in distilled water, 1602 mg/L in pH 5 buffer, 786 mg/L in pH 7 buffer, and 665 mg/L in pH 9 buffer	Memo H. Podall, 5/18/99 (D253852)
Solvent solubility (20°C for 96.6% pure sample)	0.038 mg/L in acetone 0.004 mg/L in toluene 1.11 mg/L in methanol 0.105 mg/L in isopropanol <0.003 mg/L in n-hexane	
Vapor pressure	1.00×10^{-7} torr at 20 °C	
Dissociation constant, pK_a	5.15	
Octanol/water partition coefficient	log P = -2.9 at 20 °C, pH 7	http://sitem.herts.ac.uk/aeru/footprint/en/Reports/539.htm

860.1200 Directions for Use

Currently, prohexadione calcium is registered for use on pome fruit, peanut, and grass as a plant growth regulator. BASF has proposed to register Apogee® Plant Growth Regulator (EPA Reg. No. 7969-188) for use on sweet cherries. Information pertaining to the registered/proposed end-use product is presented in Table 3. A summary of the registered/proposed use patterns is presented in Table 4.

Table 3. Summary of Registered/Proposed End-Use Products.						
Trade Name	EPA File Symbol No.	Prohexadione Calcium Concentration	Formulation Type	Target Crops	Target Pests	Label Date
Apogee® Plant Growth Regulator	7969-188	27.5%	WG	Sweet cherries	NA	7/22/10

Table 4. Summary of Proposed Directions for Use of Prohexadione Calcium.					
Applic. Timing, Type, and Equipment	Trade Name; Formulation [EPA Reg. No.]	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)
Foliar First application at shuck fall	Apogee® Plant Growth Regulator 27.5% WG [7969-188]	0.14-0.69	5	0.69	14
Use Directions and Limitations: Ground application – use dilute spray, apply to point of runoff. Aerial applications are to be made in a minimum of 10 gal/A. Use a standard fruit tree spray adjuvant, preferably a non-ionic surfactant.					

Conclusions: The submitted use directions for Apogee® Plant Growth Regulator are adequate to allow evaluation of the residue data relative to the proposed uses. However, in order to conform to the submitted field trial data, the Apogee® label should be modified to specify a PHI of 20 days.

860.1300 Nature of the Residue – Plants

Residue Chemistry Memo DP# 252547, 11/22/99, G. Kramer

Based on apple and peanut metabolism studies, the HED Metabolism Assessment Review Committee (MARC) concluded that for these crops parent compound is the only residue of concern for the tolerance expression and dietary risk assessment (Memo, G. Kramer, 4/07/00; DP# 263502). Prohexadione calcium is rapidly metabolized to prohexadione and parent-like oxidative intermediates and ultimately to tricarballylic acid, citric acid, and other natural products from the plant carbon pool. HED concludes that this decision may be translated to cherry.

860.1300 Nature of the Residue –Livestock

Data requirements regarding this guideline topic are not germane to this petition because there are no feedstuffs associated with the proposed uses on cherries.

860.1340 Residue Analytical Methods

DER Reference: 48106701.der.doc
Residue Chemistry Memo DP# 273510, 3/10/01, G. Kramer

Existing Enforcement Method: To measure residues of prohexadione calcium in plants, the petitioner developed a residue analytical method (D9601) using GC and a mass-selective detector (GC/MSD). The method was designed to measure residues of prohexadione calcium as the prohexadione methyl ester (designated by company codes BW 125-M7 or BW9054-M7). The reported level of quantitation (LOQ) for prohexadione calcium is 0.05 ppm for all apple, pear, and peanut commodities. The method was successfully validated by the Agency Analytical Chemistry Laboratories (ACL) in Fort Meade.

Proposed Enforcement Method (MRID No. 48106701): BASF Method 564/0 was developed for determination of residues of prohexadione in different plant and livestock matrices using LC-MS/MS. Prohexadione is extracted using a mixture of acetonitrile and sulfuric acid. For clean-up, an ENV+ column is used. The final determination of prohexadione is performed by LC-MS/MS. The results show that BASF method No. 564/0 is suitable to determine residues of prohexadione in the plant matrices cereal forage, grain, and straw; apple and lemon fruit, and rapeseed; as well as in the livestock matrices bovine muscle, liver, and kidney; milk; and eggs. The LOQ, defined by the lowest fortification level successfully tested, was 0.01 ppm in all sample materials. The limit of detection (LOD) was not determined.

Recovery samples, prepared at levels of LOQ and 10x LOQ in each matrix, obtained with the transition 213 → 157 (which is recommended for quantitation) were all between 70 and 120%. The method detector response was determined to be linear in the range of 0.25 to 12.5 ng/ml. The analyte standard solution was confirmed to be stable for up to 30 days under refrigeration.

The extraction solution for Method 564/0 is the same as the current enforcement methods, Method D9810 and Method D9608, which were previously radiovalidated using samples from the peanut and ruminant metabolism studies, respectively (Memo, G. Kramer, 22-NOV-1999; D252547).

BASF Method 564/0 was successfully validated by an independent laboratory, Dr. Specht & Partner Chemische Laboratorien, GMBH. However, for wheat forage, wheat straw, and rapeseed samples, significant matrix effects were observed resulting in signal suppression by about 30 to 70%. Therefore, matrix-matched external standards were used for quantitation of these matrices. Based on these results, Method 564/0 is considered suitable for data gathering in plant and livestock commodities and for enforcement purposes in apple, wheat grain, milk, egg, liver, and meat. Method 564/0 is not a suitable enforcement method for wheat forage, wheat straw, and rapeseed.

Conclusions: HED has determined that LC-MS/MS Method 564/0 is a suitable enforcement method for cherries, as defined in SOP No. ACB-019 (9/15/08).

860.1360 Multiresidue Methods

The petitioner submitted data (MRID 44457802) concerning the recovery of residues of prohexadione and its despropionyl metabolite using FDA Multiresidue Method protocols (PAM Vol. I). These data were forwarded to FDA for a complete evaluation (Memo, G. Kramer, 11/30/99; DP# 257930). The petitioner reported that residues of prohexadione and the despropionyl metabolite are not recovered using FDA Multiresidue Method protocols.

860.1380 Storage Stability

The maximum frozen storage interval from harvest to extraction for analysis was 286 days (about 9.5 months) for the sweet cherry fruit samples. Storage stability data for prohexadione calcium are available on apple fruit to support the storage conditions and interval for sweet cherry samples. The available storage stability data indicate that residues of prohexadione calcium are stable under frozen storage conditions in/on fortified apple fruit samples for at least

13 months (Memo, G. Kramer, 4/7/00; DP# 264488). These data are adequate to support the storage conditions and durations of samples from the current study.

860.1480 Meat, Milk, Poultry, and Eggs

There are no livestock feedstuffs associated with the crop addressed herein. Therefore, data requirements pertaining to meat, milk, poultry, and eggs are not relevant to this tolerance petition.

860.1500 Crop Field Trials

DER Reference: 481086703.der.doc

Eight sweet cherry field trials were conducted for prohexadione calcium (BAS 125 W) on sweet cherries encompassing North American Free Trade Agreement (NAFTA) Growing Regions 5 (MI, two trials), 10 (CA, two trials), 11 (OR, two trials; WA one trial), and 12 (OR, one trial) during the 2008 growing season.

At each trial location, one untreated control plot (Treatment 1) and one treated plot (Treatment 2) were established. The treated plot received two foliar applications of prohexadione calcium (27.5% WG formulation) at 0.338-0.346 lb ai/A (0.379-0.388 kg ai/ha), with an 11-16 day retreatment interval (RTI), for a total seasonal rate of 0.680-0.692 lb ai/A (0.762-0.776 kg ai/ha/season). The two applications were made to fruiting sweet cherry trees at 25-36 and 14-20 days prior to expected mature harvest (excluding decline trial) using ground equipment (airblast sprayers) at 61-161 gal/A of water (569-1507 L/ha). All applications were made using water as the carrier with a non-ionic surfactant adjuvant (NIS) and ammonium sulfate (AMS) in the tank mix. One untreated control and two treated sweet cherry samples from each trial were harvested at 14- to 20-day PHIs. Additionally, at one site (MI) samples were collected at a 20-day PHI, and duplicate samples of treated sweet cherry fruit were harvested at 6-, 13-, 27- and 33-day PHIs to examine residue decline.

Prohexadione calcium residues in/on sweet cherry samples (fruit) were quantified using BASF analytical method 564/0. Concurrent recoveries of prohexadione calcium fortified in control fruit samples at 0.01-5.0 ppm were 81-100% (88 ± 7%, n=6).

Prohexadione calcium residues were <0.01-0.23 ppm in/on 16 sweet cherry samples harvested at a 14- to 20-day PHI (Table 5). The residue-decline data from one trial on sweet cherries were 0.11-0.14 ppm at the 6-day PHI, 0.02 ppm at 13-day PHI, <0.01 ppm at 20-day PHI, <0.01 ppm at 27-day PHI, and <0.01 ppm at 33-day PHI, indicating that residues in/on sweet cherry fruit decline quickly with increasing PHIs.

Table 5. Summary of Residue Data from Crop Field Trials with Sweet Cherry.

Commodity; Treatment	Analyte	Total Applic. Rate (lb ai/A) [kg ai/ha]	PHI (days)	Residue Levels (ppm)[1]						
				n	Min.	Max.	HAFT[2]	Median	Mean	Std. Dev.
Sweet cherry, fruit	Prohexadione calcium	0.680-0.692 [0.762-0.776]	13-14	4	0.02	0.03	----	----	----	----
			20	14	<0.01	0.23	0.20	0.06	0.086	0.075

[1] For calculation of the HAFT, median, mean, and standard deviation, residue values of <0.01 ppm (<LOQ) were considered to be 0.01 ppm (LOQ).
[2] HAFT = highest-average field trial.

Conclusions: The submitted field trial data for sweet cherry are adequate to fulfill data requirements. The number and locations of the trials are in accordance with OPPTS Guideline 860.1500 (Table 6).

Table 6. Trial Numbers and Geographical Locations.

NAFTA Growing Zones	Cherry, sweet	
	Submitted	Requested
5	2	2
10	2	2
11	3	3
12	1	1
Total	8	8

Conclusions: The residue data for sweet cherries were entered into the OECD MRL Calculator. Based on the results of these trials, the appropriate tolerance for residues of prohexadione calcium in/on sweet cherry is 0.40 ppm. A revised Section F is requested.

860.1520 Processed Food and Feed

There is no crop associated with this petition for which HED requires residue data for processed fractions.

860.1650 Submittal of Analytical Reference Standards

An analytical standard for prohexadione calcium is currently available in the EPA National Pesticide Standards Repository (personal communication with Theresa Cole, ACB, 4/14/11); the expiration date is 2/1/19.

860.1850/860.1900 Confined/Field Accumulation in Rotational Crops

Rotational crop studies are not germane to this petition as cherries are not rotated with other crops.

860.1550 Proposed Tolerances

Tolerances are currently established under 40 CFR §180.547(a) for residues of prohexadione calcium in/on pome fruit, peanut, grass, and livestock commodities at levels ranging from 0.05 to 3.5 ppm. However, the tolerance expression for prohexadione calcium needs to be updated to reflect current Agency policy: "Tolerances are established for residues of the plant growth regulator prohexadione calcium, including its metabolites and degradates, in or on the commodities in the table below. Compliance with the tolerance levels specified below is to be determined by measuring only prohexadione calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate), in or on the following commodities:

Table 7. Tolerance Summary for Prohexadione Calcium.

Commodity	Proposed Tolerance (ppm)	HED-Recommended Tolerance (ppm)	Correct Commodity Definition
Sweet Cherries	0.50	0.40	Cherry, sweet

A revised Section F is required.

cc: G. Kramer (RAB1)
RDI: RAB1 Chemists (4/27/11)
G.F. Kramer:S10957:PY-S:(703)305-5079:7509P:RAB1
Template Version September 2005

APPENDIX I

Tolerance-Assessment Calculations

The submitted U.S. residue data for sweet cherry (8 data points) were entered into the OECD MRL Calculator in order to determine appropriate tolerance levels.

Compound	**Prohexadione Calcium**
Crop	**Sweet Cherries**
Region / Country	**USA**
GAP	**0.680-0.692 lb ai/A**

Total number of data (n)	8
Percentage of censored data	13%
Number of non-censored data	7
Lowest residue	0.010
Highest residue	0.200
Median residue	0.050
Mean	0.079
Standard deviation (SD)	0.073
Correction factor for censoring (CF)	0.917

Proposed MRL estimate

- Highest residue	0.200
- Mean + 4 SD	0.372
- CF x 3 Mean	0.217
Unrounded MRL	0.372
Rounded MRL	**0.4**

Residues (mg/kg)	n
<0.01	1
0.03	1
0.04	1
0.05	2
0.06	1
0.19	1
0.2	1

Prohexadione calcium/SFF/PC Code 112600/BASF Canada Inc./BAZ
DACO 7.4.1/7.4.2/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial – Sweet Cherry

Primary Evaluator	Sarah J. Levy, Chemist Risk Assessment Branch 1 (RAB1) Health Effects Division (HED)	Date: 02-JUN-2011
Approved by	George F. Kramer, Ph.D., Senior Chemist RAB1/HED (7509P)	Date: 02-JUN-2011

STUDY REPORT:

MRID No. 48106703. Schreier, T. (2010) Magnitude of Residue of BAS 125 in Sweet Cherry RAC after Treatment with BAS 125 11 W: Final Report. Study Identification Number: 347620; ARA-08-09-02. BASF Registration Document Number: 2010/7009172. Unpublished study prepared by BASF. 87 pages.

EXECUTIVE SUMMARY:

Eight sweet cherry field trials were conducted for prohexadione calcium (BAS 125 W) on sweet cherries encompassing North American Free Trade Agreement (NAFTA) Growing Regions 5 (MI, two trials), 10 (CA, two trials), 11 (OR, two trials; WA one trial), and 12 (OR, one trial) during the 2008 growing season.

At each trial location, one untreated control plot (Treatment 1) and one treated plot (Treatment 2) were established. The treated plot received two foliar applications of prohexadione calcium (27.5% WG formulation) at 0.338-0.346 lb ai/A (0.379-0.388 kg ai/ha), with an 11- to 16-day retreatment interval (RTI), for a total seasonal rate of 0.680-0.692 lb ai/A (0.762-0.776 kg ai/ha/season). The two applications were made to fruiting sweet cherry trees at 25-36 and 14-20 days prior to expected mature harvest (excluding decline trial) using ground equipment (airblast sprayers) at 61-161 gal/A of water (569-1507 L/ha). All applications were made using water as the carrier with a non-ionic surfactant adjuvant (NIS) and ammonium sulfate (AMS) in the tank mix. One untreated control and two treated sweet cherry raw agricultural commodity (RAC) samples (fruit) from each trial were harvested at 14- to 20-day pre-harvest intervals (PHIs). Additionally, at one site (MI) samples were collected at a 20-day PHI, and duplicate samples of treated sweet cherry fruit were harvested at 6-, 13-, 27- and 33-day PHIs to examine residue decline.

Prohexadione calcium residues in/on sweet cherry RAC samples (fruit) were quantified by liquid chromatography with tandem mass spectroscopy/mass spectroscopy (LC-MS/MS) using BASF analytical method 564/0. Acceptable concurrent recovery data for sweet cherry fruit were obtained for prohexadione calcium. Concurrent recoveries of prohexadione calcium fortified in control fruit samples at 0.01-5.0 ppm were 81-100% (88 ± 7%, n=6). Based on the lowest level of method detection (LLMV), the limit of quantitation (LOQ) was established at 0.01 ppm for prohexadione calcium and the limit of detection (LOD) was estimated at about 0.002 ppm in/on sweet cherry RAC samples.

Prohexadione calcium residues were <0.01-0.23 ppm in/on 16 sweet cherry fruit RAC samples harvested at a 14- to 20-day PHI. The residue-decline data from one trial on sweet cherries were

0.11-0.14 ppm at the 6-day PHI, 0.02 ppm at 13-day PHI, <0.01 ppm at 20-day PHI, <0.01 ppm at 27-day PHI, and <0.01 ppm at 33-day PHI, indicating that residues in/on sweet cherry fruit decline quickly with increasing PHIs.

The maximum frozen storage interval, from harvest to analysis, was 286 days (~9.5 months) for sweet cherry fruit. Previously submitted storage stability data are available to support the storage interval and conditions incurred by the samples during this study. Residues of prohexadione calcium have been shown to be stable in apple fruit stored frozen for at least 13 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document (DP# 384706).

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prohexadione calcium is a plant growth regulator that works by inhibiting the biosynthesis of gibberellins, resulting in a decrease in cell elongation and a slowing of cell growth. The chemical structure and nomenclature of prohexadione calcium is presented in Table A.1. The physicochemical properties of the technical grade of prohexadione calcium are presented in Table A.2.

TABLE A.1. Test Compound Nomenclature.

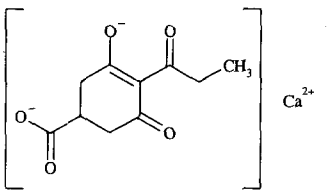
Compound	
Common name	Prohexadione calcium
Company experimental name	BAS 125 W
IUPAC name	calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate
CAS name	calcium 3-oxido-5-oxo-4-propionylcyclohexa-3-enecarboxylate
CAS registry number	127277-53-6
End-use product (EP)	Apogee® Plant Growth Regulator (EPA Reg. No. 7969-188)

TABLE A.2. Physicochemical Properties of Prohexadione Calcium.		
Parameter	Value	Reference
Water solubility (20°C for 96.6% pure sample)	174 mg/L in distilled water, 1602 mg/L in pH 5 buffer, 786 mg/L in pH 7 buffer, and 665 mg/L in pH 9 buffer	Memo H. Podall, 5/18/99 (D253852)
Solvent solubility (20°C for 96.6% pure sample)	0.038 mg/L in acetone 0.004 mg/L in toluene 1.11 mg/L in methanol 0.105 mg/L in isopropanol <0.003 mg/L in n-hexane	
Vapor pressure	1.00×10^{-7} torr at 20 °C	
Dissociation constant, pK_a	5.15	
Octanol/water partition coefficient	log P = -2.9 at 20 °C, pH 7	http://sitem.herts.ac.uk/aeru/footprint/en/Reports/539.htm

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Eight sweet cherry RAC field trials were conducted during the 2008 growing season. Each trial consisted of an untreated control plot (Treatment 1) and one treated plot (Treatment 2). The treated plot received two foliar applications of prohexadione calcium (27.5% WG) formulation of Apogee® Plant Growth Regulator, targeting 0.343 lb ai/A/application (0.385 kg ai/ha) with a 14 ± 2 day RTI. The actual rate range applied was 0.338-0.346 lb ai/A/application (0.379-0.388 kg ai/ha), with an 11- to 16-day RTI for a total rate of 0.680-0.692 lb ai/A/season (0.762-0.776 kg ai/ha/season).

The applications to fruiting sweet cherry trees (BBCH 72-87) were initiated 25-36 days prior to mature harvest, followed by the second application at 14-20 days prior to mature harvest. The resulting PHIs ranged from 14 to 20 days, excluding the decline trials. Applications were made with ground equipment typical for trees (airblast sprayers) using 61-161 gallons per acre (569-1507 L/ha), and simulated commercial treatments. All applications were made using water as the carrier and non-ionic surfactant as an adjuvant at the rate of 0.125-0.5% v/v in the final spray mixture. Also included in the tank mixture was AMS at a ratio of approximately 1:1 to the formulated Apogee® product, giving 0.31-0.34 lb AMS/acre. Sprayers were calibrated prior to each application.

The sweet cherry trial crop was grown and maintained according to typical agricultural practices for the geographical region. Typical commercial varieties of sweet cherry in established orchards were used. The weather conditions on the application days were typical and appropriate for applications for each trial site. The sites provided a range of geography, soil types, and crop variety typical of commercial production of sweet cherries. The field trials in sweet cherry were conducted according to normal agronomic practices and were not adversely impacted by environmental conditions during the trials for the geographical region. The air temperatures and rainfall for each site were generally within normal parameters, with a few fluctuations from historical means, which are expected during daily and monthly observations. The Oregon trial site had drier than normal conditions during the trials, which did not affect the established trees. Irrigation was used in the two California trials, which is typical for this

growing region. There was no major meteorological event that affected the study.

At each location, one untreated control and two treated sweet cherry RAC samples (fruit) were harvested at a 14-20 day PHI. Additionally at one site (R080295, Michigan), duplicate sweet cherry fruit samples were collected 6-, 13-, 27-, and 33-day PHI to examine the decline of prohexadione calcium residues. All samples weighed a minimum of 1 kg (2.2 lbs) and were collected from at least four individual trees. The cherry fruits were picked with stems removed in the field and pitted at the BASF Sample Preparation Laboratory to yield the RAC fruit samples. The samples were collected without bias and were commercially acceptable fruit.

TABLE B.1.1. Trial Site Conditions.

County, State or Province; Year (Trial Identification)	Soil characteristics[1]				Meteorological assessment[2]	
	Type	%OM	pH	CEC	Precipitation; Irrigation Range (inches)	Temperature (°F)
Oceana, MI; 2008 (R080295)	Spinks loamy fine sand	NR	NR	NR	1.90-5.44 (wetter)	42-48 (normal)
Allegan, MI; 2008 (R080296)	Tekenink loamy fine sand	NR	NR	NR	3.26-9.11 (wetter)	47-88 (normal)
Madera, CA; 2008 (R080297)	San Joaquin sandy loam	NR	NR	NR	0.00-0.14 Prec.; 27.5 Irrig. (normal)	50.2-91.3 (normal)
Fresno, CA; 2008 (R080298)	Hanford sandy loam	NR	NR	NR	0.00-0.14 Prec.; 12 Irrig. (normal)	50.2-91.3 (normal)
Wasco, OR; 2008 (R080299)	Wamic sandy loam	NR	NR	NR	0.00-0.00 (drier)	45-106 (normal)
Hood River, OR; 2008 (R080300)	Oak Grove loam	NR	NR	NR	0.00-0.02 (drier)	41.7-99.4 (normal)
Klickitat, WA; 2008 (R080301)	Wind River sandy loam	NR	NR	NR	0.00-0.23 (normal)	41.7-99.4 (normal)
Plok, OR; 2008 (R080302)	Woodburn silt loam	NR	NR	NR	0.42-0.49 (slightly drier)	46-71 (normal)

[1] NR = not reported, OM = organic matter, CEC = cation-exchange capacity in meq/100 g soil.
[2] Data are for the interval of the month of first application through the month of harvest.

TABLE B.1.2. Study Use Pattern.

Location County, State or Province; Year (Trial ID)	EP[1]	Method; Timing (growth stage)	Volume[2] (gal/A) [L/ha]	Rate (lb ai/A) [kg ai/ha]	RTI[3] (days)	Total Rate (lb ai/A) [kg ai/ha]	Adjuvants[4]
Oceana, MI; 2008 (R080295)	27.5% WG	Foliar; BBCH73-75	60.82 [569]	0.345 [0.386]	11	0.689 [0.771]	NIS 0.25% v/v
		Foliar; BBCH78-81	134.6 [1259]	0.344 [0.385]			
Allegan, MI; 2008 (R080296)	27.5% WG	Foliar; BBCH75-76	73.83 [690]	0.340 [0.381]	11	0.680 [0.762]	NIS 0.25% v/v
		Foliar; BBCH81-87	161.2 [1507]	0.340 [0.381]			
Madera, CA; 2008 (R080297)	27.5% WG	Foliar; BBCH81	100.8 [942]	0.346 [0.388]	14	0.691 [0.774]	Helena COC; NIS 0.5% v/v
		Foliar; BBCH84	100.3 [939]	0.345 [0.386]			

13

TABLE B.1.2. Study Use Pattern.

Location County, State or Province; Year (Trial ID)	EP[1]	Method; Timing (growth stage)	Volume[2] (gal/A) [L/ha]	Rate (lb ai/A) [kg ai/ha]	RTI[3] (days)	Total Rate (lb ai/A) [kg ai/ha]	Adjuvants[4]
Fresno, CA; 2008 (R080298)	27.5% WG	Foliar; BBCH81	100.9 [943]	0.346 [0.388]	14	0.692 [0.776]	Helena COC
		Foliar; BBCH84	100.8 [942]	0.346 [0.388]			
Wasco, OR; 2008 (R080299)	27.5% WG	Foliar; Fruit ½ final size	116.8 [1092]	0.345 [0.386]	14	0.688 [0.770]	Regulaid
		Foliar; Straw colored fruit	127 [1187]	0.343 [0.384]			
Hood River, OR; 2008 (R080300)	27.5% WG	Foliar; Pit hardened	109 [1019]	0.346 [0.388]	14	0.684 [0.767]	Regulaid
		Foliar; Advanced fruit coloring	101.2 [946]	0.338 [0.379]			
Klickitat, WA; 2008 (R080301)	27.5% WG	Foliar; Fruit ½ final size	115.6 [1081]	0.342 [0.383]	14	0.688 [0.771]	Regulaid
		Foliar; Straw colored fruit	126 [1178]	0.346 [0.388]			
Plok, OR; 2008 (R080302)	27.5% WG	Foliar; BBCH72	120.0 [1122]	0.343 [0.384]	16	0.689 [0.772]	R-11
		Foliar; BBCH79	123.7 [1157]	0.346 [0.388]			

[1] EP = end-use product; Apogee® formulation contains 27.5% of prohexadione calcium.
[2] All applications of prohexadione calcium were made using water as the carrier.
[3] RTI = retreatment interval.
[4] A NIS typically used for fruit trees was applied at the label rate (typically 0.05-0.5%) in the spray mixture. An AMS was also applied in all trials at 0.30-0.36 lbs/A as a tank-mix.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Zones	Cherry, sweet	
	Submitted	Requested
5	2	2
10	2	2
11	3	3
12	1	1
Total	8	8

B.2. Sample Handling and Preparation

The sweet cherry fruit samples were transferred within 4 hours to freezers on the date of harvest, maintained frozen during storage, and shipped within 103 days by freezer truck to the analytical laboratory, BASF Agro Research (Research Triangle Park, NC). All samples were received from the field by BASF Agro Research were frozen and determined to be in good condition prior to homogenization for analysis.. The sweet cherry fruit samples were pitted and homogenized with dry ice to a consistency appropriate for analysis. The homogenized samples were shipped frozen to the BASF analytical laboratory in Guaratinguetá, São Paulo, Brazil for analysis. Samples were stored frozen in plastic containers until the time of analysis.

B.3. Analytical Methodology

The sweet cherry RAC samples were analyzed for prohexadione calcium residues using a previously validated LC-MS/MS method, entitled "Method for the Determination of Prohexadione-Ca in Plant and Animal Matrices, 564/0." This method was also successfully validated concurrently with the sweet cherry analyses.

Briefly, prohexadione calcium residues in sweet cherry samples were extracted with acetonitrile (ACN): sulfuric acid solution and cleaned-up on a ENV+ column eluted with washes of 1% formic acid, and final elution with methanolic formic acid. High-performance liquid chromatography (HPLC) using a C18 column was used to isolate the prohexadione analyte and with MS/MS detection in the positive-ionization mode was used to monitor ion transitions from *m/z* 213→157 for prohexadione. A molecular weight conversion factor (1.179x) was used to express prohexadione acid as parent prohexadione calcium equivalents. The method LOD and validated LOQ for residues of prohexadione calcium in/on sweet cherry fruit were 0.002 and 0.01 ppm, respectively. Analytical data such as supporting raw data necessary for re-calculations, representative chromatograms, a description of minor modifications to the method for the analysis of sweet cherry, and example calculations were provided.

C. RESULTS AND DISCUSSION

Sample storage conditions and durations are summarized in Table C.2. All RAC samples were frozen within 4 hours of collection and maintained frozen during storage and transport to the sample preparation and analysis facility. All samples were received frozen from the field and were stored in a freezer at BASF Agro Research prior to pitting, homogenization, and analysis. The maximum frozen storage interval from harvest to extraction for analysis was 286 days (about 9.5 months) for the sweet cherry fruit samples. Storage stability data for prohexadione calcium are available on apple fruit to support the storage conditions and interval for sweet cherry samples. The available storage stability data indicate that residues of prohexadione calcium are stable under frozen storage conditions in/on fortified apple fruit samples for at least 13 months (Memo, G. Kramer, 07-APR-2000; D264488). These data are adequate to support the storage conditions and durations of samples from the current study.

The performance of the analytical method on sweet cherries was evaluated during each sample set by fortifying controls with a standard solution containing the reference substance. Concurrent recoveries of prohexadione calcium fortified into control sweet cherry fruit samples at 0.01, 0.5, or 5.0 ppm ranged 81-100% (average 88 ± 7%; n=6). The method used was adequately validated in conjunction with the field sample analyses (Table C.1). The method LOQ was established at 0.01 ppm by recovery of prohexadione calcium through the clean-up method and detection with the LC-MS/MS. The LOD was estimated to be approximately 0.002 ppm based upon 20% of the LOQ and detection at 3-5-times the signal:noise ratio.

The results of the sweet cherry crop field trials are reported in Table C.3, and a summary of the residue data is presented in Table C.4. The analytical results for the treated samples from the eight trials show that after two applications at 0.338-0.346 lb ai/A (0.379 to 0.388 kg ai/ha), the prohexadione calcium residues were <0.01-0.23 ppm in 16 treated samples harvested at 14-20

days PHI. Residues of prohexadione calcium of 0.02 ppm and <0.01 ppm were found in/on the two untreated control sweet cherry RAC samples from California. However, residues of prohexadione calcium in the treated samples from these two trials were not corrected for residues found in the untreated samples, since residues in the associated controls were near the method LOQ. The results from the decline trial show that the residues of prohexadione calcium were 0.11 and 0.14 ppm at 6-days PHI, decreased to 0.02 ppm at 13-days PHI, and subsequently declined below the LOQ (<0.01 ppm) at 20-, 27-, and 33-days PHI.

TABLE C.1. Summary of Concurrent Recoveries of Prohexadione Calcium from Sweet Cherry.

Matrix	Analyte	Spike Level (ppm)	Sample Size (n)	Recoveries (%)	Mean ± Std. Dev. (%)
Sweet cherry, fruit	Prohexadione calcium	0.01	2	91, 100	96 ± 7
		0.5	2	83, 84	84 ± 1
		5.0	2	81, 87	84 ± 5
	Overall	**0.01-5.0**	**6**	**81-100**	**88 ± 7**

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)[1]	Actual Storage Duration[2]	Limit of Demonstrated Storage Stability
Sweet cherry, fruit	<-0	~9.5 months	13 months[3]

[1] The storage temperature is from the time of sample receipt at the analytical facility until analysis.
[2] The actual study duration is the time from field sampling through pitting, homogenization and analysis.
[3] Residues are stable for 13 months in/on fortified apple fruit samples (Memo, G. Kramer, 07-APR-2000; D264488).

TABLE C.3. Residue Data from Crop Field Trials with Prohexadione Calcium.

County, State or Province; Year (Trial ID)	Zone	Crop/ Variety	Matrix	Form.	Method; Timing (growth stage)	Total Rate (lb ai/A) [kg ai/ha]	PHI (days)	Prohexadione calcium Residues (ppm)
Oceana, MI; 2008 (R080295)	5	Cherry, sweet/ Sams Golden	Fruit	27.5% WG	Foliar; BBCH73-75 or BBCH78-81	0.689 [0.771]	6	0.11
							6	0.14
							13	0.02
							13	0.02
							20	<0.01
							20	<0.01
							27	<0.01
							27	<0.01
							33	<0.01
							33	<0.01
Allegan, MI; 2008 (R080296)	5	Cherry, sweet/ Hedelfinger	Fruit	27.5% WG	Foliar; BBCH75-76 or BBCH81-87	0.680 [0.762]	14	0.03
							14	0.03
Madera, CA; 2008 (R080297)	10	Cherry, sweet/ Brooks	Fruit	27.5% WG	Foliar; BBCH81 or BCH84	0.691 [0.774]	20	0.23
							20	0.17
Fresno, CA; 2008 (R080298)	10	Cherry, sweet/ Brooks	Fruit	27.5% WG	Foliar; BBCH81 or BBCH84	0.692 [0.776]	20	0.20
							20	0.18

16

TABLE C.3. Residue Data from Crop Field Trials with Prohexadione Calcium.

County, State or Province; Year (Trial ID)	Zone	Crop/ Variety	Matrix	Form.	Method; Timing (growth stage)	Total Rate (lb ai/A) [kg ai/ha]	PHI (days)	Prohexadione calcium Residues (ppm)
Wasco, OR; 2008 (R080299)	11	Cherry, sweet/ Lapin	Fruit	27.5% WG	Foliar; Fruit ½ final size or straw colored fruit	0.688 [0.770]	20	0.02
							20	0.06
Hood River, OR; 2008 (R080300)	11	Cherry, sweet/ Skeena	Fruit	27.5% WG	Foliar; Pit hardened or advance fruit coloring	0.684 [0.767]	20	0.04
							20	0.06
Klickitat, WA; 2008 (R080301)	11	Cherry, sweet/ Lapin	Fruit	27.5% WG	Foliar; Fruit ½ final size or straw colored fruit	0.688 [0.771]	20	0.05
							20	0.07
Plok, OR; 2008 (R080302)	12	Soybean/ Royal Ann	Fruit	27.5% WG	Foliar; BBCH72 or BBCH79	0.689 [0.772]	20	0.05
							20	0.05

[1] When calculating combined residues, the LOQ (0.01 ppm for each analyte) was used for values reported as <LOQ.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Sweet Cherry.

Commodity; Treatment	Analyte	Total Applic. Rate (lb ai/A) [kg ai/ha]	PHI (days)	Residue Levels (ppm)[1]						
				n	Min.	Max.	HAFT[2]	Median	Mean	Std. Dev.
Sweet cherry, fruit	Prohexadione calcium	0.680-0.692 [0.762-0.776]	13-14	4	0.02	0.03	----	----	----	----
			20	14	<0.01	0.23	0.20	0.06	0.086	0.075

[1] For calculation of the HAFT, median, mean, and standard deviation, residue values of <0.01 ppm (<LOQ) were considered to be 0.01 ppm (LOQ).
[2] HAFT = highest-average field trial.

D. CONCLUSION

The results from the eight crop field trials on sweet cherry show that after two applications of prohexadione calcium, each targeted at 0.343 lb ai/A (0.385 kg ai/ha) for a seasonal total of about 0.69 lb ai/A (0.77 kg ai/ha), the maximum residue was 0.23 ppm in/on sweet cherry fruit samples harvested at a 20-day PHI. The HAFT residue level was 0.20 ppm. The residue decline data from the one Michigan trial demonstrate that residues of prohexadione calcium in sweet cherry fruit decline with increasing PHI.

An acceptable method was used for quantitation of residues in/on sweet cherry commodities. Adequate storage stability data are available to support sample storage durations and conditions.

E. REFERENCES

DP#s: 264488 & 264533
Subject: PP# 8F04941. Prohexadione-Calcium, in/on Peanuts, Pome Fruits, and Cattle Meat Byproducts (Kidney). **Review of Amendments Dated 2/18/00 & 3/16/00 Submitted in Response to HED's Memo of 11/22/99.** Revised Sections B & F and Storage Stability Data.

From: G.F. Kramer
Dated: 07-APR-2000
MRIDs: 45067901

F. DOCUMENT TRACKING

RDI: RAB1 Chemists (27-APR-2011)
Petition Number: 0F7765
DP#: 384706
PC Code: 112600

Template Version June 2005.

18

Prohexadione Calcium/SFF/PC Code 118203/BASF Canada Inc./BAZ
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method - Plant

Primary Evaluator	George F. Kramer, Ph.D., Senior Chemist Risk Assessment Branch 1 (RAB1) Health Effects Division (HED)	Date: 02-JUN-2011
Approved by	Dana M. Vogel, Branch Chief RAB1, HED	Date: 02-JUN-2011

STUDY REPORTS:

MRID No. 48106701. A. Lehmann and C. Mackenroth. (2005) Validation of the Analytical Method 564/0: Method for the Determination of Prohexadione-Ca in Plant and Animal Matrices. BASF Registration Document No. 2005/1007581. Unpublished study prepared by BASF Corporation, Agricultural Products. 65 pages.

MRID No. 48106702. Thomas Anspach. (2005) Independent Laboratory Validation of BASF Method 564/0: Method for the Determination of Prohexadione-Ca in Plant and Animal Matrices (BASF Study Code 134612). BASF Registration Document No. 2005/1006484. Unpublished study prepared by BASF Corporation, Agricultural Products. 59 pages.

EXECUTIVE SUMMARY:

BASF Method 564/0 was developed for determination of residues of prohexadione in different plant and livestock matrices using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Prohexadione is extracted using a mixture of acetonitrile and sulfuric acid. For clean up, an ENV+ column is used. The final determination of prohexadione is performed by LC-MS/MS. The results show that BASF method No. 564/0 is suitable to determine residues of prohexadione in the plant matrices cereal forage, grain, and straw; apple and lemon fruit, and rapeseed; as well as in the livestock matrices bovine muscle, liver, and kidney; milk; and eggs. The limit of quantitation (LOQ), defined by the lowest fortification level successfully tested, was 0.01 ppm in all sample materials. The limit of detection (LOD) was not determined.

Recovery samples, prepared at levels of LOQ and 10x LOQ in each matrix, obtained with the transition 213 → 157 (which is recommended for quantitation) were all between 70 and 120%. The method detector response was determined to be linear in the range of 0.25 to 12.5 ng/ml. The analyte standard solution was confirmed to be stable for up to 30 days under refrigeration.

The extraction solution for Method 564/0 is the same as the current enforcement methods, Method D9810 and Method D9608, which were previously radiovalidated using samples from the peanut and ruminant metabolism studies, respectively (Memo, G. Kramer, 22-NOV-1999; D252547).

BASF Method 564/0 was successfully validated by an independent laboratory, Dr. Specht & Partner Chemische Laboratorien, GMBH. However, for wheat forage, wheat straw, and rapeseed samples, significant matrix effects were observed resulting in signal suppression by about 30 to 70%. Therefore, matrix-matched external standards were used for quantitation of these matrices. Based on these results, Method 564/0 is considered suitable for data gathering in

plant and livestock commodities and for enforcement purposes in apple, wheat grain, milk, egg, liver, and meat. Method 564/0 is not a suitable enforcement method for wheat forage, wheat straw, and rapeseed.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, analytical method test data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP# 384706].

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and No Data Confidentiality statements were provided. No deviations from regulatory requirements were noted in the provided studies that would affect the validity of the study.

A. BACKGROUND INFORMATION

Prohexadione calcium is a plant growth regulator that works by inhibiting the biosynthesis of gibberellins, resulting in a decrease in cell elongation and a slowing of cell growth. The chemical structure and nomenclature of prohexadione calcium is presented in Table A.1. The physicochemical properties of the technical grade of prohexadione calcium are presented in Table A.2.

TABLE A.1. Test Compound Nomenclature.

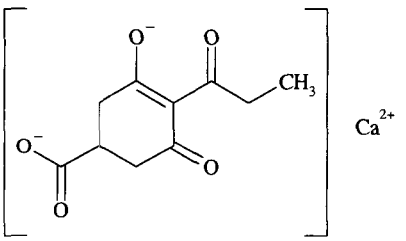
Compound	
Common name	Prohexadione calcium
Company experimental name	BAS 125 W
IUPAC name	calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate
CAS name	calcium 3-oxido-5-oxo-4-propionylcyclohexa-3-enecarboxylate
CAS registry number	127277-53-6
End-use product (EP)	Apogee® Plant Growth Regulator (EPA Reg. No. 7969-188)

TABLE A.2. Physicochemical Properties of Prohexadione Calcium.		
Parameter	Value	Reference
Water solubility (20°C for 96.6% pure sample)	174 mg/L in distilled water, 1602 mg/L in pH 5 buffer, 786 mg/L in pH 7 buffer, and 665 mg/L in pH 9 buffer	Memo H. Podall, 5/18/99 (D253852)
Solvent solubility (20°C for 96.6% pure sample)	0.038 mg/L in acetone 0.004 mg/L in toluene 1.11 mg/L in methanol 0.105 mg/L in isopropanol <0.003 mg/L in n-hexane	
Vapor pressure	1.00 x 10^{-7} torr at 20 °C	
Dissociation constant, pK_a	5.15	
Octanol/water partition coefficient	log P = -2.9 at 20 °C, pH 7	http://sitem.herts.ac.uk/aeru/footprint/en/Reports/539.htm

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Prohexadione is extracted using a mixture of acetonitrile and sulfuric acid. For clean up an ENV+ column is used. The final determination of prohexadione is performed by LC-MS/MS (figure 1, copied from MRID No. 48106701).

21

Figure 1: Flow Chart of Method 564/0

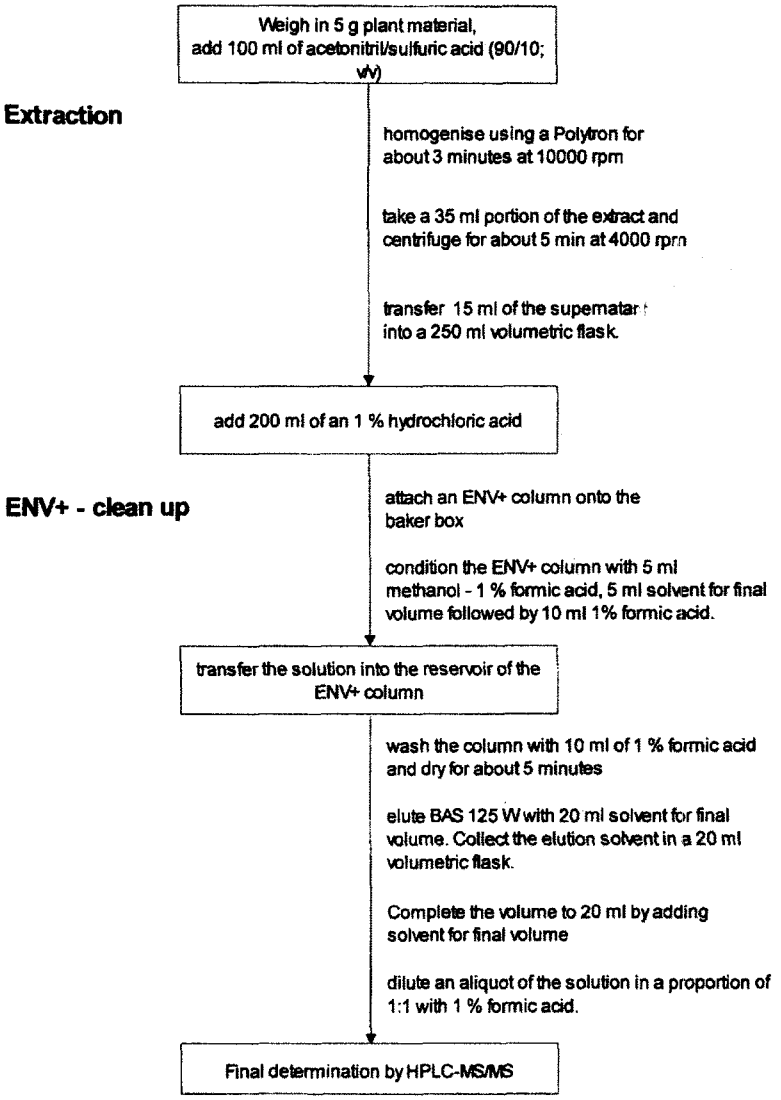


TABLE B.1. Summary Parameters for the Analytical Method Used for Quantitation of Prohexadione Calcium Residues in Plants and Livestock.	
Method ID	BASF Method 564/0
Analyte(s)	Prohexadione Calcium
Extraction solvent/technique	Acetonitrile/2M sulfuric acid, 90/10, v/v
Cleanup strategies	ENV+ column eluted with acetonitrile/methanol/formic acid, 95/5/1, v/v/v.
Instrument/Detector	Instrument: PE API 4000 Mass Spectrometer HPLC System: Agilent 1100 LC Binary Pump Column: Betasil C18 100*2.1 mm 5 µm The transitions monitored for prohexadione were 213 → 157 (quantitation) and 213 → 139 (confirmation).
Standardization method	External standardization using a calibration curve of prohexadione. The standard curve is obtained by direct injection of the standard into LC-MS/MS in the range of 0.25 to 12.5 ng/ml.
Stability of std solutions	Stable in acetonitrile/methanol/formic acid for 1 month in refrigerator.
Retention times	~4.1 to 5.6 minutes.

B.2. Enforcement Method

The enforcement method is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

The results show that BASF Method No. 564/0 is suitable to determine residues of prohexadione in the plant matrices cereal forage, grain, and straw; apple and lemon fruit, and rapeseed; as well as in the livestock matrices bovine muscle, liver, and kidney; milk; and eggs. The mean results for each fortification level obtained with the transition 213 → 157 (which is recommended for quantitation) were all well between 70 and 120%. In case of transition 213 → 139, the two matrices cereal straw and apple yielded maximum recoveries of 123 and 126%, respectively. The relative standard deviations (RSD, %) for all commodities were below 20%. The LOQ, defined by the lowest fortification level successfully tested, was 0.01 ppm in all sample materials. The LOD was not determined. The method recoveries are presented in Table C.1.1. The characteristics of the data-gathering method are presented in Table C.1.2. Chromatograms for untreated control samples and fortified control samples were provided, showing no significant interferences for any of the analytes.

Extraction Efficiency

The extraction solution for Method 564/0 is the same as the current enforcement methods, Method D9810 and Method D9608, which were previously radiovalidated using samples from the peanut and ruminant metabolism studies, respectively (Memo, G. Kramer, 22-NOV-1999; D252547).

TABLE C.1.1. Recovery Results from Method Validation Using the Data-Gathering Analytical Method (copied from MRID No. 48106701).

Matrix	Fort. level [mg/kg]	Transition 213 → 157 * Recoveries (%)					mean [%]	SD [+/-]	CV [%]	Transition 213 → 139 Recoveries (%)					mean [%]	SD [+/-]	CV [%]
Plant matrices																	
Cereal forage	0.01	80.3	82.6	95.8	107.3	84.2	90.0	11.3	12.6	83.6	70.4	82.8	91.0	76.5	80.9	7.8	9.6
	0.1	81.9	87.5	94.8	89.1	88.8	88.4	4.6	5.2	81.0	84.5	93.3	86.0	82.5	85.4	4.8	5.6
						Overall mean:	89.2	8.2	9.2					Overall mean:	83.1	6.6	7.9
Cereal grain	0.01	105.5	105.3	101.4	101.0	111.6	105.0	4.3	4.1	95.5	91.7	95.3	95.8	94.4	94.5	1.7	1.8
	0.1	94.0	90.7	95.0	96.0	96.5	94.4	2.3	2.5	95.0	91.2	92.6	95.9	94.9	93.9	1.9	2.1
						Overall mean:	99.7	6.4	6.4					Overall mean:	94.2	1.7	1.8
Cereal straw	0.01	81.5	82.3	88.3	80.6	85.2	83.6	3.2	3.8	123.3	118.5	128.5	128.9	117.3	123.3	5.4	4.4
	0.1	97.9	98.4	97.5	93.7	95.5	96.6	2.0	2.0	96.9	98.3	99.1	94.3	96.3	96.8	1.9	2.0
						Overall mean:	90.1	7.3	8.1					Overall mean:	110.0	14.5	13.2
Apple fruit	0.01	100.3	100.1	108.9	105.0	103.9	103.6	3.6	3.5	128.7	124.3	127.5	125.1	121.7	125.5	2.8	2.2
	0.1	96.1	95.2	91.7	91.8	91.3	93.6	3.0	3.2	98.4	98.8	95.0	94.3	94.4	96.2	2.2	2.3
						Overall mean:	98.6	6.1	6.2					Overall mean:	110.8	15.6	14.1
Lemon fruit	0.01	76.4	91.5	84.5	86.0	72.1	82.1	7.8	9.5	86.9	106.4	98.4	96.5	95.3	97.3	6.0	6.2
	0.1	96.1	93.5	90.9	91.2	89.0	92.2	2.7	3.0	89.9	94.6	90.7	92.1	97.7	93.0	3.2	3.4
						Overall mean:	87.1	7.6	8.8					Overall mean:	95.1	5.1	5.3
Oilseed rape seed	0.01	80.3	85.4	82.6	81.9	89.0	83.9	3.4	4.1	85.9	84.0	77.7	79.5	91.5	83.7	5.5	6.5
	0.1	85.4	85.5	84.4	80.9	83.7	84.0	1.9	2.3	83.0	84.7	83.4	82.5	81.4	83.0	1.2	1.5
						Overall mean:	83.9	2.6	3.1					Overall mean:	83.4	3.8	4.5
Animal matrices																	
Bovine muscle	0.01	96.5	97.0	92.1	100.5	99.6	97.1	3.3	3.4	86.0	91.4	100.1	91.9	94.4	92.8	5.1	5.5
	0.1	100.5	69.0	87.2	99.4	98.8	91.0	13.4	14.7	98.7	70.0	84.4	98.0	98.7	90.0	12.7	14.1
						Overall mean:	94.1	9.8	10.4					Overall mean:	91.4	9.2	10.1
Bovine liver	0.01	73.7	78.7	81.7	79.2	80.7	78.8	3.1	3.9	81.8	102.7	75.9	83.8	84.4	85.7	10.1	11.7
	0.1	79.7	81.5	79.1	80.7	85.6	81.3	2.6	3.1	80.6	84.1	79.8	79.3	86.1	82.0	3.0	3.6
						Overall mean:	80.1	3.0	3.7					Overall mean:	83.8	7.3	8.7
Bovine kidney	0.01	79.3	82.7	80.2	79.2	77.9	79.9	1.8	2.2	79.5	70.3	66.9	75.2	72.6	72.9	4.8	6.6
	0.1	102.5	102.1	100.1	102.8	98.2	101.1	2.0	1.9	93.6	98.5	94.1	92.9	98.6	95.5	2.8	2.9
						Overall mean:	90.5	11.4	12.6					Overall mean:	84.2	12.5	14.8
Milk	0.01	99.5	100.2	107.4	106.7	105.8	103.9	3.8	3.6	101.0	101.6	103.4	107.2	109.2	104.5	3.6	3.4
	0.1	96.0	98.5	94.7	94.9	101.7	97.2	2.9	3.0	96.6	97.5	97.0	94.9	99.1	97.0	1.5	1.6
						Overall mean:	100.5	4.8	4.8					Overall mean:	100.8	4.7	4.7
Egg	0.01	93.8	93.7	103.0	100.5	104.3	99.1	5.0	5.1	93.9	105.0	89.8	97.5	99.6	97.1	5.8	5.9
	0.1	96.3	96.3	96.0	98.1	97.6	96.9	1.0	1.0	96.6	97.0	100.0	98.1	97.2	97.8	1.3	1.4
						Overall mean:	98.0	3.6	3.7					Overall mean:	97.5	4.0	4.1

* used for quantitation

TABLE C.1.3. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Prohexadione Calcium Residues in Plants and Livestock.	
Analyte(s)	Prohexadione Calcium
Equipment ID	Instrument: PE API 4000 Mass Spectrometer HPLC System: Agilent 1100 LC Binary Pump Column: Betasil C18 100*2.1 mm 5 µm
LOQ	0.01 ppm for all matrices tested.
LOD	Not reported.

TABLE C.1.3. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Prohexadione Calcium Residues in Plants and Livestock.	
Accuracy/Precision	Adequate recoveries (70-120%) within each fortification level obtained from transition 213 → 157 with acceptable RSD (<20). This demonstrated acceptable accuracy/precision of the LC-MS/MS method.
Reliability of the Method [ILV]	An independent laboratory method validation [ILV], Study No. 134612, was conducted to verify the reliability of Method 564/0. These results indicated that LC-MS/MS Method 564/0 is reliable.
Linearity	The concentration/response was linear in the range 0.25 to 12.5 ng/ml.
Specificity	The control chromatograms have no peaks above the chromatographic background near the analyte peak of interest. The spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. Two transitions were monitored for each analyte/matrix for quantitation and confirmation purposes.

C.2. Enforcement Method

The current enforcement methods, Method D9810 and Method D9608, for plant and livestock commodities, respectively, were described previously (Memo, G. Kramer, 22-NOV-1999; D252547). The proposed enforcement method for cherries, Method 564/0, is the same as the data-gathering method.

C.3. Independent Laboratory Validation

An independent laboratory method validation (ILV) was conducted by Dr. Specht & Partner Chemische Laboratorien, GMBH. Untreated control samples were spiked with prohexadione calcium at 0.01 ppm and 0.1 ppm. Calculation of results was based on peak area measurements using a calibration curve. The standard curve was obtained by injecting seven external standard solutions of prohexadione covering the working range of 0.100-10.0 ng/mL. The final determination was performed by LC-MS/MS. For apple, wheat grain, milk, egg, liver, and meat samples no significant matrix effects were observed. Therefore, solvent-matched external standards were used for quantitation of these matrices. For wheat forage, wheat straw, and rapeseed samples, more significant matrix effects were observed resulting in signal suppression by about 30 to 70%. Therefore, matrix-matched external standards were used for quantitation of these matrices. Based on these results, Method 564/0 is considered suitable for data-gathering in plant and livestock commodities and for enforcement purposes in apple, wheat grain, milk, egg, liver, and meat. Method 564/0 is not a suitable enforcement method for wheat forage, wheat straw, and rapeseed.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Prohexadione Calcium Residues in Plants and Livestock (copied from MRID No. 48106702).

Matrix	Forti-fication level [mg/kg]	Recoveries Prohexadione-Ca* single values [%]	mean [%]	Std. dev. [%]	Rel. std. dev. [%]	No. of analyses	Overall recovery mean [%]	Std. dev. [%]	Rel. std. dev. [%]
Apple	0.01	83, 85, 85, 79, 89	84	3.6	4.3	5	85	2.7	3.2
	0.1	83, 85, 85, 87, 86	85	1.5	1.8	5			
Wheat forage***	0.01	98, 99, 102, 98, 107	101	3.8	3.8	5	103	4.5	4.4
	0.1	99, 108, 108, 107, 107	106	3.8	3.6	5			
Wheat grain	0.01	88, 94, 99, 94, 99	95	4.5	4.7	5	92	4.6	5.0
	0.1	85, 95, 91, 89, 90	90	3.6	4.0	5			
Wheat straw***	0.0	107, 99, 98, 101, 101	101	3.5	3.5	5	94	8.7	9.3
	0.1	94, 82, 91, 87, 81	87	5.6	6.4	5			
Oil seed rape***	0.0	105, 109, 98, 104, 100	103	4.3	4.2	5	103	3.8	3.7
	0.1	99, 105, 99, 107, 104	103	3.6	3.5	5			
Milk	0.0	92, 94 105, 139**, 96	97	5.7	5.9	5	100	4.9	4.9
	0.1	103, 102, 102, 99, 106	102	2.5	2.5	5			
Egg	0.0	104, 104, 97, 100, 88	99	6.6	6.7	5	98	4.6	4.7
	0.1	97, 96, 96, 100, 97	97	1.6	1.6	5			
Liver	0.0	97, 90, 87, 82, 98	91	6.8	7.5	5	84	9.2	11
	0.1	79, 78, 71, 79, 74	76	3.6	4.7	5			
Beef	0.0	93, 94, 83, 83, 92	89	5.5	6.2	5	95	7.7	8.1
	0.1	99, 102, 98, 103, 105	101	2.9	2.9	5			

* ... Measured as Prohexadione but re-calculated for Prohexadione-Ca

** ... Discarded as outlier due to the Grubbs test

***... Due to significant matrix effects quantitation was performed by matrix-matched external standards

D. CONCLUSION

BASF Method 564/0 was developed for determination of residues of prohexadione in different plant and livestock matrices using LC-MS/MS. The results show that BASF method No. 564/0 is suitable to determine residues of prohexadione in the plant matrices cereal forage, grain, and straw; apple and lemon fruit, and rapeseed; as well as in the livestock matrices bovine muscle, liver, and kidney; milk; and eggs. BASF Method 564/0 was successfully validated by an independent laboratory, Dr. Specht & Partner Chemische Laboratorien, GMBH. However, for wheat forage, wheat straw, and rapeseed samples, significant matrix effects were observed resulting in signal suppression by about 70 to 30%. Therefore, matrix-matched external standards were used for quantitation of these matrices. Based on these results, Method 564/0 is considered suitable for data-gathering in plant and livestock commodities and for enforcement purposes in apple, wheat grain, milk, egg, liver, and meat. Method 564/0 is not a suitable enforcement method for wheat forage, wheat straw, and rapeseed.

E. REFERENCES

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F. DOCUMENT TRACKING

RDI: RAB1 Chemists (4/27/11).
Petition Number: 0F7765
DP#: 384706
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Chemical Name: Prohexadione calcium

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